



King Saud University
Arabian Journal of Chemistry

www.ksu.edu.sa
www.sciencedirect.com



ORIGINAL ARTICLE

Controlling contamination for determination of ultra-trace levels of priority pollutants chlorophenols in environmental water matrices

Yassine Kadmi ^{a,*}, Lidia Favier ^{a,*}, Tania Yehya ^b, Isabelle Soutrel ^a,
Andrei Ionuț Simion ^c, Christophe Vial ^b, Dominique Wolbert ^a

^a *Ecole Nationale Supérieure de Chimie de Rennes, CNRS, UMR 6226, 11, Allée de Beaulieu, CS 50837, 35708 Rennes Cedex 7, Université Européenne de Bretagne, France*

^b *Clermont Université, Université Blaise Pascal, Institut Pascal, 24 Avenue des Landais, BP 20206, 63174 Aubière Cedex, CNRS, UMR 6602, IP, 63171 Aubière, France*

^c *“Vasile Alecsandri” University of Bacău, Faculty of Engineering, Department of Chemical and Food Engineering, 157 Calea Mărășești, 600115 Bacău, Romania*

Received 16 February 2015; accepted 3 June 2015

KEYWORDS

Water quality;
Chlorophenols;
Extraction;
Liquid chromatography

Abstract Recently, environmental scientists have been focused their attention on the occurrence of emerging contaminants in water, such as disinfection by products (DBPs), including chlorophenols. These pollutants can be a public health problem due to their carcinogenic properties. In this work, ultra-high pressure liquid chromatography (UHPLC) coupled to a photodiode array detector (PDA) was used for the development of an analytical method capable of simultaneous identification

Abbreviations: UHPLC/PDA, ultra-high pressure liquid chromatography coupled to a photodiode array detector; SPE, solid phase extraction; CPs, chlorinated phenols; 2-CP, 2-chlorophenol; 2,4-DCP, 2,4-dichlorophenol; ATP, adenosine triphosphate; MAC, maximum admissible concentration; CE, capillary electrophoresis; LC, liquid chromatography; GC, gas chromatography; FID, flame-ionization detection; MS, mass spectrometry detection; SPME, solid-phase microextraction; SDME, single drop microextraction; LOD, limit of detection; LOQ, limit of quantification; RSD, relative standard deviation; RE, accuracy (relative error); R, extraction recoveries

* Corresponding authors. Tel.: +33 (0)2 23 23 81 34; fax: +33 (0)2 23 23 80 20.

E-mail addresses: yassine.kadmi@gmail.com (Y. Kadmi), lidia.favier@ensc-rennes.fr (L. Favier).

Peer review under responsibility of King Saud University.



Production and hosting by Elsevier

<http://dx.doi.org/10.1016/j.arabjc.2015.06.005>

1878-5352 © 2015 Production and hosting by Elsevier B.V. on behalf of King Saud University.

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Please cite this article in press as: Kadmi, Y. et al., Controlling contamination for determination of ultra-trace levels of priority pollutants chlorophenols in environmental water matrices. Arabian Journal of Chemistry (2015), <http://dx.doi.org/10.1016/j.arabjc.2015.06.005>

and quantification of two chlorophenols namely, 2-chlorophenol and 2,4-dichlorophenol in water samples. In addition, a solid phase extraction (SPE) procedure for the extraction of these compounds was optimized. The chlorophenols were separated by an Acquity BEH C18 (100×2.1 mm, $1.7 \mu\text{m}$) column with a mobile phase of acetonitrile/ultrapure water/formic acid (55/45/0.1, v/v/v). The flow rate of the mobile phase was 0.4 mL min^{-1} . The optimized SPE–UHPLC/PDA technique was evaluated in terms of robustness, considering the enrichment factor for all of the studied chlorophenols. Linear calibration was obtained with correlation coefficients $r^2 \geq 0.998$. Intra-day and inter-day precision was less than 5% and accuracy ranged from 99.95% to 103.32%, respectively. The obtained extraction recoveries were higher than 98%. The pre-concentration factor was 2.500 for the both analytes. Under optimized conditions, the detection limits of the overall SPE–UHPLC/PDA method were in the ng L^{-1} level. The excellent performance of the developed method, as well as the short analysis time makes it a promising analytical tool for the screening of chlorophenols in environmental water samples.

© 2015 Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Chlorinated phenols (CPs) belong to the most important environmental contaminants. They are commonly used in the production of paper and pesticides but also as intermediates in the production of dyes, plastics and pharmaceuticals (Li et al., 2001; Santana et al., 2007; de Morais et al., 2012). These kinds of applications often lead to wastewater and groundwater contamination. In addition, this class of organic compounds has been also detected in drinking water as a result of tap water chlorination treatment (Jin and Yang, 2006; Jung and Son, 2008; Gurzau et al., 2010; Kadmi et al., 2014a,b).

According to different studies, chlorophenols represent a public health concern due to their estrogenic, mutagenic or carcinogenic effects (Wada et al., 1999; Michalowicz and Duda, 2007; Gavrilescu, 2009). They are highly toxic, interfering with oxidative phosphorylation and inhibiting ATP synthesis, poor biodegradable and present recalcitrant properties (González et al., 2010; Anbia et al., 2012; Cozma et al., 2012; Sarafraz-Yazdi et al., 2012). Their toxicity depends on the degree of chlorination and the position of chlorine atoms in relation to the hydroxyl group and pH (Czaplicka, 2004; Ho et al., 2008). Thus, almost of these molecules have been included in the priority pollutants list by US Environmental Protection Agency (USEPA) (Wennrich et al., 2000) and the European Union (EU) legislation (Bagheri et al., 2004a). In addition, taking into account their toxicity many countries and international organizations have limited their maximum concentration in drinking water (Padilla-Sánchez et al., 2011; Elci et al., 2011). For example, the European Union legislation (EU Directive 2455/2001/EC) has set a maximum admissible concentration (MAC) of total phenols in drinking water to $0.5 \mu\text{g L}^{-1}$ and $0.1 \mu\text{g L}^{-1}$ for individual compounds (Peng et al., 2007; Elci et al., 2011). Chlorophenols are generally present at trace levels in water. The most often detected CPs in water are 2-chlorophenol (2-CP) and 2,4-dichlorophenol (2,4-DCP). Their concentration is very variable and depends on the type of water. For example, in wastewaters the detected values ranged from ng L^{-1} to $\mu\text{g L}^{-1}$ (Quintana et al., 2007; Regueiro et al., 2009) and even to mg L^{-1} (Limam et al., 2010). In freshwaters and drinking waters more variable levels have been observed (from low $\mu\text{g L}^{-1}$ to low ng L^{-1} range) (Perret et al., 2004; Kawaguchi et al., 2005; Michalowicz and Duda, 2007; Elci et al., 2011).

Therefore, for all these reasons, the need for monitoring and controlling the presence of CPs in the aqueous environment is now well recognized being crucial for achieving good water-quality objectives. As a consequence, there is an increased interest for the development of easily operating detection methods for the monitoring of these molecules in order to provide a complete overview of their occurrence in water samples.

Several analytical techniques including capillary electrophoresis (CE) (Ali and Aboul-Enein, 2002; Puig et al., 2008) or liquid chromatography (LC) (Cass et al., 2000; Wennrich et al., 2000; Jin and Yang, 2006), particularly reverse liquid chromatography in combination with UV, fluorescence, electrochemical or mass spectrometry detection (Kawaguchi et al., 2005) have been proposed in the past for the determination of phenols and phenolic compounds derivatives (Quintana and Ramos, 2008). Gas chromatography (GC) analysis, using several detection methods such as flame-ionization detection (FID), electron-capture detection (ECD) or mass spectrometry detection (MS) was the most often used because of its high separation efficiency and low limits of quantification. However, for such analysis, a derivatization step is most often necessary in order to improve the chromatographic performance and sensitivity of the selected compounds requiring more time and effort (Chung and Lee, 2008; Quintana and Ramos, 2008). Liquid–liquid extraction (LLE), solid-phase microextraction (SPME) and single drop microextraction (SDME) methods were developed and used for pre-concentration of chlorophenols (Bagheri et al., 2004b; Conosa et al., 2005; Quintana and Ramos, 2008). Some of these techniques are time consuming, expensive or require high volumes of hazardous organic solvents. For this reason, in the field of environmental analysis is an increasing tendency to use solid phase extraction (SPE) for the clean-up and pre-concentration of liquid samples. The SPE–HPLC combined with PDA for determination of phenols compounds was used in the past. However, the obtained data demonstrated that the detection limits are significantly higher (Patsias and Papadopoulou-Mourkidou, 2000; Li et al., 2007; Opeolu et al., 2007).

In the present paper, we proposed an analytical methodology based on an efficient pre-concentration step by solid phase extraction (SPE) followed by an ultra-high pressure liquid chromatography with photodiode array detection

(UHPLC/PDA) for the simultaneous determination of 2-chlorophenol and 2,4-dichlorophenol at ultra-trace levels in water samples. Rapidity, high enrichment factor, high extraction recovery and simplicity of operation are some of the main advantages of the proposed analytical strategy. The obtained results demonstrated that the processed methodology is very useful for the screening of the target analytes in water samples. To our knowledge, this is the first work reporting the analysis of these molecules at ultra-trace levels in French real waters.

2. Experimental

2.1. Reagents and samples

All the reagents and solvents used in this study were of the highest analytical purity grade. The studied compounds include 2-chlorophenol (2-CP, 99%) and 2,4-dichlorophenol (2,4-DCP, 99%) were purchased from Sigma Aldrich GmbH (Steinheim, Germany). The main physico-chemical properties of these molecules are presented in Table 1.

Acetonitrile and formic acid were purchased from J.T. Baker (Deventer, The Netherlands). Methanol and dichloromethane were obtained from Fischer Scientific-Bioblock (Illkirch, France). Acetic acid and ethyl acetate were supplied by Acros Organics (Noisy-le-Grand, France).

2.2. Preparation of standard solutions and water samples

Individual stock standard solutions of 1000 mg L⁻¹ of each chlorophenol were prepared in methanol. A stock standard mixture of the target compounds was weekly prepared by diluting the individual stock solutions in methanol. The working solutions were freshly prepared in acetonitrile/ultrapure water (55/45, v/v) by appropriate dilutions of the stock standard solution to reach the working concentration range. This composition ensured good stability of the chlorophenols in water samples. The ultrapure water used for the preparation of the samples was produced by an Elga Option-Q DV-25 system (Antony, France). All solutions were stored in glass bottles in the dark at -20 °C. Standard mixtures were preserved two months and working solutions three weeks. To demonstrate the applicability of the developed method real water samples including river water and drinking water were used in this work. The samples were collected in October 2013 and January 2014, from different locations (from Brittany region, France). All samples were collected in baked glass 10 L amber bottles with Teflon lined caps to ensure sample integrity, filtered through a 0.45 µm cellulose membrane and then stored in the dark at 4 °C until their analysis (within one week of collection).

2.3. Apparatus and analytical conditions

The quantification of chlorophenols was achieved using an Acquity™ ultra-high pressure liquid chromatography system

coupled to a photodiode array detector (UHPLC/PDA), purchased from Waters (Saint-Quentin en Yvelines, France).

The chromatographic analyses were carried out using an Acquity UHPLC H-Class system, containing a binary pump, an auto-sampler and a thermostated column compartment (Waters, Saint-Quentin en Yvelines, France). Chromatographic conditions that directly affect chromatographic separation such as chromatographic column, elution mode, mobile phase composition and additives, temperature and flow rate were studied and optimized in this work. Separation of chlorophenols was carried out using an Ethylene Bridged Hybrid (BEH) C18 column (100 × 2.1 mm, 1.7 µm; Waters, Ireland). The column in the chromatographic system was protected by an in-line filter unit purchased from Waters (Saint-Quentin en Yvelines, France). The analytical column compartment was maintained at 45 °C. The auto-sampler was conditioned at 5 °C. The flow rate was maintained at 0.4 mL min⁻¹ and the injection volume was set to 5 µL. Acetonitrile (A) and ultrapure water (B) (55/45 v/v) containing 0.1% (v/v) formic acid (pH = 5) were used as mobile phase. Elution was done under isocratic mode. Under optimized analytical conditions the total run time was 4.5 min. In addition, blanks were periodically run during the analysis to confirm the absence of contamination. Details on the optimized UHPLC conditions are presented in Section 3.1. Furthermore, the detection wavelength was optimized. The PDA scanning range was between 200 and 350 nm. Multiple wavelength UV detection was used for the quantification of analytes: 275 nm for 2-CP and 286 nm for 2,4-DCP. The Empower 2 software (Waters, Saint-Quentin en Yvelines, France) was employed to control the instruments, data acquisition and processing.

2.4. Sample preparation

The sample extraction procedure was performed by using an off-line SPE 12-port Visiprep SPE vacuum manifold obtained from Supelco (Bellefonte, PA, USA). Oasis HLB (Hydrophilic-Lipophilic Balanced) cartridges (6 cc, 200 mg, Waters, Milford, MA) were selected in this study for the pre-concentration of analytes. Recovery experiments were designed to evaluate the elution efficiency of different organic solvents for conditioning step, loading rates and elution conditions.

After the extraction step, the organic eluent was collected in a 14 mL, conical graduated glass Pyrex® tube (VWR, Fontenay-sous-Bois, France). The resulting extracts were evaporated in a water bath at 30 °C and concentrated under a high-purity nitrogen stream using an N-Evap system (Organomation, Berlin, MA, USA). Finally, the obtained extracts were reconstituted using acetonitrile/ultrapure water (55/45, v/v).

2.5. Quality parameters

2.5.1. Linearity, limit of detection and limit of quantification

The linearity of the method was studied from the calibration curves prepared from spiked ultrapure water samples at seven

Table 1 Selected physico-chemical proprieties of target compounds.

Compound	Abbreviation	Molecular formula	CAS no.	Molecular weight (g mol ⁻¹)	Log <i>K</i> _{ow}	p <i>K</i> _a
2-Chlorophenol	2-CP	ClC ₆ H ₄ OH	95-57-8	128.5	3.15	8.44
2,4-Dichlorophenol	2,4-DCP	Cl ₂ C ₆ H ₃ OH	120-83-2	163.0	3.06	7.89

concentrations of chlorophenols ranging from 25 to 200 $\mu\text{g L}^{-1}$. Each solution was analyzed in triplicate. The calibration curves were plotted by the peak area versus the concentration of analyte. The linearity was evaluated by linear regression analysis determined by the least squares regression method. This method was used to determine the slope, intercept, and correlation coefficient (r^2) of the linear regression equation. The instrumental limit of detection (IDL) is the lowest concentration of analyte that the analytical process can reliably differentiate from background levels, while the instrumental limit of quantification (IQL) is the lowest concentration of analyte that can be quantified. The IDL and the IQL values were estimated at concentrations with a signal-to-noise ratio (S/N) of 3 and 10, respectively.

2.5.2. Precision and accuracy

The precision of the proposed instrumental method was evaluated in terms of repeatability (intra-day and inter-day precision). The repeatability values were expressed as relative standard deviation (RSD, %). The accuracy (RE, %) was expressed by the following:

$$RE, \% = \frac{\text{Mean of Observed Concentration}}{\text{Spiked Concentration}} \cdot 100 \quad (1)$$

Moreover, the RSD calculated at each concentration level was not allowed to exceed 15% and the RE had to be within $\pm 15\%$ of the actual value.

2.5.3. Extraction recovery

SPE recoveries were determined quantitatively at low and high concentration levels. The extraction recoveries (R, %) were calculated using the following procedure: A sample spiked with the analytes was extracted using the developed solid phase extraction procedure and the analysis result was compared to that of an unextracted standard which was prepared at the equivalent final concentration. So, the extraction recoveries were calculated as the ratio between the resulting peak areas of the extracted and non-extracted samples. In order to evaluate the efficiency of the proposed method in monitoring of low levels of chlorophenols, their levels in ultrapure and real (river) water samples were investigated. Then, the relative standard deviations (RSD, %) were calculated.

3. Results and discussion

3.1. Optimization of UHPLC/PDA conditions

The primary aim of this work was to evaluate the potential of UHPLC system for the analysis of chlorophenols in water samples. The UHPLC system takes full advantage of chromatographic separation with high resolution and rapid analysis time by using columns packed with smaller particles (1.7 μm). It is well known, that the type of separation column, mobile phase composition, column temperature and flow rate applied during analysis affect the elution of analytes. Indeed, these three parameters, greatly affect the peak shapes, analysis times and thus the chromatographic resolution. It will be noticed, that these parameters were optimized here. These experiments were carried out before the SPE process.

Three types of UHPLC columns, Acquity BEH HSS T3 (100 \times 2.1 mm, 1.7 μm), Acquity BEH C8 (100 \times 2.1 mm,

1.7 μm) column and Acquity BEH C18 column (100 \times 2.1 mm, 1.7 μm) were tested to improve the UHPLC separation of the target analytes. The obtained results showed that C18 column provides a better retention and separation than Acquity BEH HSS T3 column. On the other hand, we compared C8 column with Acquity C18 column, and it was found that C18 column is better for capturing chlorophenols than C8. A C18 column would theoretically provide a better retention of 2-CP and 2,4-DCP than a C8 column, because longer chain lengths may be more appropriate for retention of small compounds. The high retention on C18 made chlorophenols eluted out at higher organic portions in mobile phase. Therefore, their determination is improved, where the signal intensities increased with the portion of organic modifier in mobile phase. Hence, from the view of sensitivity, C18 showed the best performance and it was chosen for this work.

Generally in chromatographic analysis, the separation of analytes is largely dependent on the solvent conditions. Therefore, the effects of mobile phase composition and of additives on sensitivity and separation of the target chlorophenols were also investigated. In this study, different compositions of binary mixtures of acetonitrile, methanol and water were tested as eluting solvents in both isocratic and gradient modes to achieve good separation in minimum run time for all target analytes. A good compromise between high peak shapes and a reasonably analysis time was obtained with acetonitrile/water and with an isocratic elution mode. So, this phase was selected as mobile phase in this work (data not shown). Furthermore, in order to achieve an efficient separation of chlorophenols by using BEH C18 column, we tested and compared acetonitrile/water containing formic acid with acetonitrile/water containing acetic acid by changing their percentage in ultrapure water at 0.05%, 0.1%, 0.2%, 0.3% and 0.4% (data not shown).

The obtained results indicated that the retention of the chlorophenols is affected by the pH of the mobile phase. In addition it was observed that the measured responses using acetonitrile/water, modified with 0.1% formic acid were higher than those using acetonitrile/water containing acetic acid (Fig. 1). Indeed, it is known that the pH of the mobile phase affects the retention of phenols in the column, depending on the degree of dissociation. Partial dissociation could lead to peak broadening and asymmetric peaks due to co-elution of the acid and appearance of its conjugate base. As a consequence, the acidification of the aqueous mobile phase has a favorable effect on the separation, as the dissociation of analytes, retention times are shorter and peak asymmetry is improved (Mahugo Santana et al., 2009). For this reason the separation was performed at low pH and acetonitrile/water modified with 0.1% formic acid was selected as mobile phase due to the good separation and high sensitivity for all compounds. In general, the retention time increases with the concentration in formic acid in solvent A. Therefore, this additive was kept at 0.1% to ensure satisfactory quantification.

The effect of column temperature and flow rate was also studied. Different column temperatures from 35 $^{\circ}\text{C}$ to 50 $^{\circ}\text{C}$ were evaluated and it was observed that a temperature of 45 $^{\circ}\text{C}$ significantly improves the resolution, peak shape, intensity of the response and retention times of the considered analytes.

Effect of flow rates from 0.2 to 0.5 mL min^{-1} on the responses of 2-CP and 2,4-DCP was also tested. Results

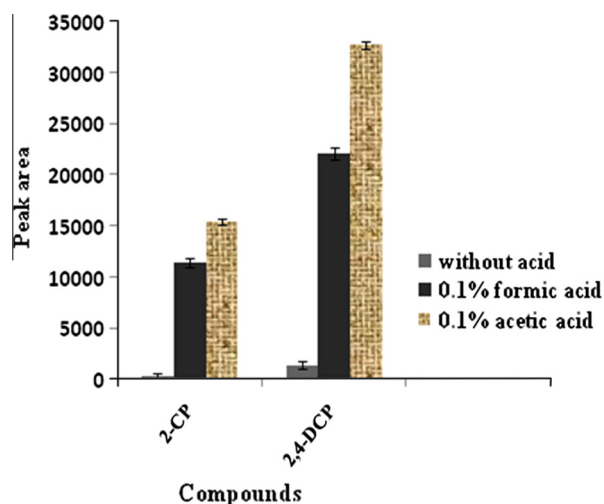


Figure 1 Influence of the presence of acetic acid and formic acid in mobile phase (acetonitrile/ultrapure water (55:45, v/v)) on the chlorophenols measured responses. The mobile phase was modified with 0.1% formic acid and 0.1% acetic acid in water.

showed that the flow rate significantly affects the signal intensity and therefore the peak area (Fig. 2). It can be observed that the peak areas of chlorophenols are enhanced by the increasing of the flow rate and reached a maximum value when the flow rate was 0.4 mL min^{-1} . Subsequently, it decreases gradually with the increasing of the flow rate. This effect is supposed to be due to the instability of chlorophenols at elevated flow rates.

Therefore, 0.4 mL min^{-1} was selected as the working flow rate in this study. Under these experimental conditions, the target analytes were eluted rapidly (the retention time of the last eluting peak $< 3 \text{ min}$) and the separation and resolution of peaks were good. The obtained retention times for 2-CP and 2,4-DCP were 1.35 min and 2.56 min, respectively (Fig. 3). It should be noted that under this analytical conditions the UHPLC separation was six times faster than the conventional HPLC separation with the same analytes. Moreover, it consumes ca. 50% less organic solvents than the corresponding HPLC technique (Li et al., 2007; Opeolu et al., 2007). Finally, the results indicated that optimized UHPLC method developed here for the analysis of

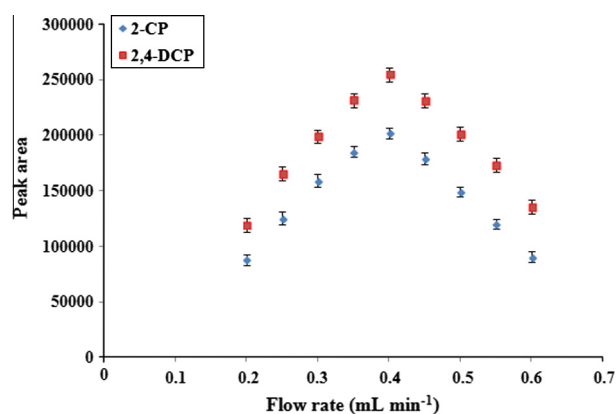


Figure 2 Effect of flow rate on the peak area of chlorophenols.

chlorophenols has other practical advantages such as faster analysis times and reduced solvent consumption rates, compared to conventional HPLC technique.

Since a PDA was used here for the detection of analytes, the absorption wavelengths were also optimized in order to reach the maximum sensitivity for each compound. The wavelengths selected for the simultaneous detection of chlorophenols were: 275 nm for 2-CP and 286 nm for 2,4-DCP. Therefore, this analytical UHPLC/PDA technique can be considered as a promising method that can easily compete with conventional HPLC/PDA systems in this field of application.

3.2. Optimization of SPE process

In order to determine lower concentrations of chlorophenols in water samples, a pre-concentration step is necessary. As stated below in order to decrease the limit of detection and quantification of these analytes an off-line-SPE methodology was developed in this work. SPE experiments were carried out after the optimization of UHPLC/PDA conditions. The optimization of the extraction process was performed in order to attain excellent recoveries for all target compounds in a single extraction step. According to literature data, Oasis HLB cartridges are one of the most used, because they are able to retain a large list of organic pollutants through its unique ratio of hydrophilic N-vinylpyrrolidone and lipophilic divinylbenzene sorbent (de Almeida et al., 2000). Thus, Oasis HLB cartridges were selected for this work.

Different parameters of the extraction method, such as organic solvents for conditioning step, loading rates and elution conditions, were investigated and optimized to determine the operating conditions providing the highest recovery in ultrapure water samples. Consequently, the optimal operating

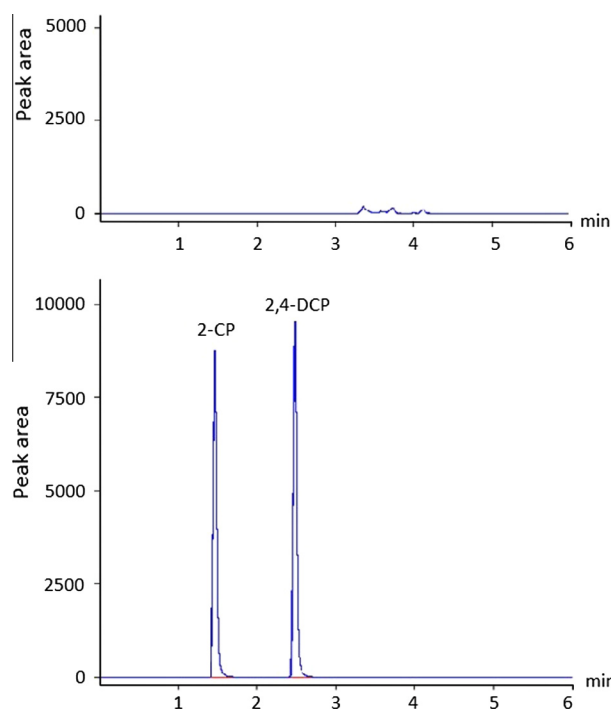


Figure 3 The obtained retention times for 2-2-chlorophenol (2-CP) and 2,4 dichlorophenol (2,4-DCP).

conditions for the SPE method consisted of the use of acetonitrile and methanol (6 mL of each) for conditioning the HLB sorbent. Generally, in the SPE methodology, the flow rate of the sample affects the retention of the target molecules on the surface of the adsorbent.

The optimum flow rate used for the extraction of the samples was determined to be between 3 and 5 mL min⁻¹, and there was no effect on recovery. In addition, several organic solvents of variable polarity were tested as eluent solvents, including acetonitrile, ethyl acetate, dichloromethane and methanol (data not shown). Finally, methanol was selected as the most effective solvent for the elution step.

Therefore, the optimized SPE method is established as follows: Oasis HLB cartridges are conditioned using 2 × 3 mL acetonitrile, and 2 × 3 mL methanol. The SPE cartridges were equilibrated with 2 × 5 mL of ultrapure water acidified with formic acid (pH 3). All compounds were spiked into the 250 mL water sample. The sample passed through the SPE cartridges at a flow rate of 4 mL min⁻¹. After the extraction, the cartridges were washed with 2 × 3 mL of ultrapure water adjusted to pH 3 with formic acid. Then, the molecules adsorbed onto the sorbents were eluted successively with 2 × 2 mL of methanol at a flow rate of 3 mL min⁻¹. The eluates were transferred to a clean tube and concentrated by evaporation under a nitrogen stream to a final volume of approximately 0.1 mL (concentration factor of 2500). The obtained extracts are then reconstituted using acetonitrile/ultrapure water (55/45, v/v) and transferred to injection vials. Finally, the extracts were stored at 4 °C until further analysis.

3.3. Analytical performance

The performance characteristics of the proposed method were established by determining the linearity, limit of detection, limit of quantification, precision, accuracy, and extraction recovery.

3.3.1. Linearity, working range, limit of detection, and limit of quantification

The linearity of instrumental method was investigated by direct injection using calibration solutions at seven concentration levels, ranging from 25 to 1000 µg L⁻¹. Excellent linearity with high correlation coefficients ($r^2 \geq 0.998$) was observed. The developed UHPLC/PDA method was linear up to 1000 µg L⁻¹ for 2-CP and 2,4-DCP, respectively. The linear regression equations of the calibration curves for 2-CP and 2,4-DCP were:

$$y = 22.5x - 45.3 \quad (2)$$

and

$$y = 24.1x - 30.7 \quad (3)$$

where y represents the peak area and x represents the concentration of the analyte. The instrumental detection limit (IDL) of 2-CP and 2,4-DCP was 25 µg L⁻¹ and 30 µg L⁻¹, respectively. Moreover, the instrumental quantification limit (IQL) of 2-CP and 2,4-DCP was found to be 30 µg L⁻¹ and 60 µg L⁻¹, respectively. These results indicate that our UHPLC/PDA method (without the pre-concentration) is not enough sensitive for analysis of major chlorophenols in drinking water samples. Thus, a pre-concentration step is needed in order to improve its sensitivity.

3.3.2. Precision and accuracy

Intra-day and inter-day studies for precision and accuracy of the proposed instrumental method (UHPLC/PDA) were carried out by direct injection of a solution containing the two phenolic compounds ($n = 6$) at two concentration levels for each analyte (60 and 600 µg L⁻¹). The obtained intra-day precisions (RSD) for all compounds were below 3.67% and calculated inter-day RSD were lower than 4.78% (Table 2). The intra- and inter-day accuracy results ranged from 99.95% to 103.32%, respectively. Therefore, the RSD values were lower than 15% indicating high precision of the developed method. The detailed data of intra-day, inter-day precision and accuracy values are given in Table 2. The presented results clearly demonstrate that the developed UHPLC/PDA method is precise and accurate.

3.3.3. Extraction recovery

As stated before, a simple method based on SPE was developed and optimized for the extraction of the 2-CP and 2,4-DCP. The performance of the proposed SPE protocol was investigated through extraction recoveries obtained for six replicates in spiked ultrapure water samples at two concentrations (30 and 60 ng L⁻¹). Examination of results obtained by using the optimized SPE procedure showed very good recoveries for 2-CP and 2,4-DCP (Table 3). Indeed, the calculated extraction recoveries for 2-CP and 2,4-DCP were within the range of 99.50–100.1% and 98.80–99.70%, respectively. Moreover, the RSD values ranged from 3.15% to 4.28% at the quality control levels (Table 3). On the other hand, the instrumental detection limits of the developed method were 25 µg L⁻¹ and 30 µg L⁻¹ for 2-CP and 2,4-DCP, respectively, while the method detection limits (MDL) obtained with the overall SPE–UHPLC/PDA procedure were 10 ng L⁻¹ for 2-CP and 12 ng L⁻¹ for 2,4-DCP, respectively. These results are better than those previously reported in the literature for SPE–LC analysis (Li et al., 2007; Opeolu et al., 2007). Moreover, these values are below the legal tolerance level for

Table 2 Intra-day, inter-day precision and accuracy values of the instrumental method in ultrapure water.

Compounds	Conc. spiked (µg L ⁻¹)	Intra-day ($n = 6$)		Inter-day ($n = 6$)	
		Precision (RSD, %)	Accuracy (RE, %)	Precision (RSD, %)	Accuracy (RE, %)
2-CP	60	3.67	99.95	4.32	100.05
	600	4.13	101.55	4.65	102.12
2,4-DCP	60	3.19	100.14	4.11	102.87
	600	4.24	100.33	4.78	103.32

Table 3 Extraction recoveries (%) and relative standard deviations (RSD, %) of chlorophenols using SPE step.

Analytes	Conc. spiked (ng L ⁻¹)	Conc. measured (ng L ⁻¹)	Recovery (%)	RSD (% , n = 6)
2-CP	30	29.85	99.50	3.15
	60	60.06	100.1	3.93
2,4-DCP	30	29.64	98.80	4.28
	60	59.82	99.70	4.06

Table 4 Analytical results of river water samples using the developed SPE–UHPLC/PDA method.

Analytes	Mean concentration (ng L ⁻¹)	Conc. spiked (ng L ⁻¹)	Conc. measured (ng L ⁻¹)	Recovery (%)	RSD (% , <i>n</i> = 6)
<i>Water sample 1</i>					
2-CP	n.d. ^a	30	29.85	99.50	3.15
		60	60.06	100.1	3.93
2,4-DCP	n.d. ^a	30	29.64	98.80	4.28
		60	59.82	99.70	4.06
<i>Water sample 2</i>					
2-CP	n.d. ^a	30	30.15	100.5	5.03
		60	61.14	101.9	4.93
2,4-DCP	n.d. ^a	30	30.12	100.4	4.28
		60	61.68	102.8	4.97
<i>Water sample 3</i>					
2-CP	n.d. ^a	30	29.73	99.10	4.11
		60	60.30	100.5	4.65
2,4-DCP	n.d. ^a	30	29.19	97.30	5.13
		60	59.16	98.60	4.94

^a n.d. referred to not detected.

each chlorophenol in drinking water (500 ng L⁻¹) according to the European Community Directive (Peng et al., 2007; Elci et al., 2011). The proposed SPE–UHPLC/PDA method therefore allowed quantification limits in the range of ng L⁻¹ and an enrichment factor of 2500 for the both phenolic compounds (sample volume, 250–0.1 mL).

3.4. Application of the proposed method: analysis of real water samples

In order to investigate the applicability of the proposed SPE–UHPLC/PDA method, the extraction and determination of the target analytes were conducted under the optimum conditions using real environmental water samples, including river waters. Three water samples were collected in October 2013 and January 2014 from different locations situated in the Brittany region (France) and analyzed using the SPE–UHPLC/PDA. Furthermore, the analytes were not detected in the real environmental waters (Table 4). The river samples ($n = 6$) were firstly spiked with the standard solutions of chlorophenols at two concentration levels in order to assess the matrix effects. The results obtained for extraction recoveries and standard deviations are listed in Table 4. Extraction recoveries higher than 97% were obtained for all compounds from all river water samples, with RSD values < 6%. These satisfactory results demonstrate that the matrices of the real water samples analyzed here have no significant effects for the determination of chlorophenols by the developed SPE–UHPLC/PDA method.

The potential applicability of the proposed method was also evaluated by analyzing drinking water samples from four locations in France. They were collected in triplicates from each location and analyzed. The results demonstrated that the collected samples were free of contamination with 2-CP and 2,4-DCP (data not shown). The analysis of real water samples showed that the resulting CPs levels posed no health risk for the population of the Brittany region at the time of sampling.

The overall results presented in this work confirm that the combined use of SPE protocol and UHPLC/PDA technique is suitable for the simultaneous determination of 2-CP and 2,4-DCP in ultrapure water, environmental water and drinking water samples.

4. Conclusions

The present study demonstrates that it is possible to increase the analytical sensitivity of priority pollutants such as chlorophenols by coupling UHPLC technique with a PDA detection system and a SPE procedure. A new method for the pre-concentration and simultaneous quantification of 2-CP and 2,4-DCP in different types of water samples has been developed. Good linearity, precision, accuracy, lower limits of detection and limits of quantification were obtained. The used Oasis HLB cartridges lead to high recoveries and to achieve a 2500-fold concentration factor for the both analytes. In addition, the values of the calculated extraction recoveries were satisfactory compared with those from the previous

studies. The combined SPE procedure and UHPLC/PDA technique is a rapid, low-cost, and reliable method for the routine analysis of 2-CP and 2,4-DCP at ultra-trace concentration levels (ng L^{-1}) in different types of water samples. In addition, in this method the consumption of toxic organic solvents is also minimized. The validation allowed us to control various analytical aspects to certify the reliability of the method like the measurement of uncertainty.

To the best of our knowledge, this work provides the first information regarding the occurrence of chlorophenols in environmental water and in drinking water samples from the Brittany region (France). In conclusion, the presented methodology can be a helpful analytical tool to evaluate the environmental pollution risk of these hazardous organic pollutants even at ng L^{-1} levels.

References

- Ali, I., Aboul-Enein, H.Y., 2002. Determination of phenol and its derivatives in waste water by capillary electrophoresis. *Fresen. Environ. Bull.* 11, 36–39.
- Anbia, M., Haghi, A., Shariati, S., 2012. Novel fiber coated with nanoporous carbons for head space solid-phase microextraction of chlorophenols from aqueous media. *Anal. Method* 4, 2555–2561.
- Bagheri, H., Mohammadi, A., Salemi, A., 2004a. On-line trace enrichment of phenolic compounds from water using a pyrrole-based polymer as the solid-phase extraction sorbent coupled with high-performance liquid chromatography. *Anal. Chim. Acta* 513, 445–449.
- Bagheri, H., Saber, A., Mousavi, S.R., 2004b. Immersed solvent microextraction of phenol and chlorophenols from water samples followed by gas chromatography–mass spectrometry. *J. Chromatogr. A* 1046, 27–33.
- Cass, Q.B., Freitas, L.G., Foresti, E., Damianovic, M.H.R., 2000. Development of HPLC method for the analysis of chlorophenols in samples from anaerobic reactors for wastewater treatment. *J. Liq. Chromatogr. R.T.* 23, 1089–1097.
- Chung, L.W., Lee, M.R., 2008. Evaluation of liquid-phase microextraction conditions for determination of chlorophenols in environmental samples using gas chromatography–mass spectrometry without derivatization. *Talanta* 76, 154–160.
- Conosa, P., Rodriguez, I., Rubi, E., Cela, R., 2005. Optimization of solid phase microextraction conditions for the determination of triclosan and possible related compounds in water samples. *J. Chromatogr. A* 1072, 107–115.
- Cozma, P., Hlihor, R.M., Apostol, L.C., Diaconu, M., Pogăcean, M.O., Gavrilăscu, M., 2012. Aerobic biodegradation of phenol by activated sludge in a batch reactor. *Environ. Eng. Manage. J.* 11, 2053–2058.
- Czaplicka, M., 2004. Sources and transformations of chlorophenols in the natural environment. *Sci. Total Environ.* 322, 21–39.
- de Almeida, A.D., Lacorte, S., Vinas, T., Viana, P., Barcelo, D., 2000. Monitoring of priority pesticides and other organic pollutants in river water from Portugal by gas chromatography–mass spectrometry and liquid chromatography–atmospheric pressure chemical ionization mass spectrometry. *J. Chromatogr. A* 879, 13–26.
- de Moraes, P., Stoichev, T., Basto, M.C.P., Vasconcelos, M.T.S.D., 2012. Extraction and pre-concentration techniques for chromatographic determination of chlorophenols in environmental and food samples. *Talanta* 89, 1–11.
- Elci, L., Kolbe, N., Elci, S.G., Anderson, J.T., 2011. Solid phase extractive pre-concentration coupled to gas chromatography–atomic emission detection for the determination of chlorophenols in water samples. *Talanta* 85, 551–555.
- Gavrilăscu, M., 2009. Behaviour of persistent pollutants and risks associated with their presence in the environment – integrated studies. *Environ. Eng. Manage. J.* 8, 1517–1531.
- Gurza, A.E., Popovici, E., Pinte, A., Dumitrăscu, I., Pop, C., Popa, O., 2010. Exposure assessment to trihalomethanes from the epidemiological perspective. *Carpathian J. Earth Environ. Sci.* 6 (1), 5–12.
- González, L.F., Sarria, V., Sánchez, O.F., 2010. Degradation of chlorophenols by sequential biological-advanced oxidative process using *Trametes pubescens* and TiO_2/UV . *Bioresour. Technol.* 101, 3493–3499.
- Ho, H.P., Lee, R.J., Lee, M.R., 2008. Purge-assisted headspace solid-phase microextraction combined with gas chromatography–mass spectrometry for determination of chlorophenols in aqueous samples. *J. Chromatogr. A* 1213, 245–248.
- Jin, M., Yang, Y., 2006. Simultaneous determination of nine trace mono- and di-chlorophenols in water by ion chromatography atmospheric pressure chemical ionization mass spectrometry. *Anal. Chim. Acta* 566, 193–199.
- Jung, C.W., Son, H.J., 2008. The relationship between disinfection by-products formation and characteristics of natural organic matter in raw water. *Korean. J. Chem. Eng.* 25, 714–720.
- Kadmi, Y., Favier, L., Soutrel, I., Lemasle, M., Wolbert, D., 2014a. Ultra-trace-level determination of N-nitrosodimethylamine, N-nitrosodiethylamine, and N-nitrosomorpholine in waters by solid-phase extraction followed by liquid chromatography–tandem mass spectrometry. *Cent. Eur. J. Chem.* 12, 928–936.
- Kadmi, Y., Favier, L., Wolbert, D., 2014b. N-nitrosamines, emerging disinfection by-products of health concern: an overview of occurrence, mechanisms of formation, control and analysis in water. *Water Sci. Technol.* 15, 11–25.
- Kawaguchi, M., Ishii, Y., Sakui, N., Okanouchi, N., Ito, R., Saito, K., Nakazawa, H., 2005. Stir bar sorptive extraction with in situ derivatization and thermal desorption–gas chromatography–mass spectrometry for determination of chlorophenols in water and body fluid samples. *Anal. Chim. Acta* 533, 57–65.
- Li, D., Park, J., Oh, J.R., 2001. Silyl derivatization of alkylphenols, chlorophenols, and bisphenol A for simultaneous GC/MS determination. *Anal. Chem.* 73, 3089–3095.
- Li, J., Zhao, X., Shi, Y., Cai, Y., Mou, S., Jiang, G., 2007. Mixed hemimicelles solid-phase extraction based on cetyltrimethylammonium bromide-coated nano-magnets Fe_3O_4 for the determination of chlorophenols in environmental water samples coupled with liquid chromatography/spectrophotometry detection. *J. Chromatogr. A* 1180, 24–31.
- Limam, I., Guenne, A., Driss, M.H., Mazéas, L., 2010. Simultaneous determination of phenol, methylphenols, chlorophenols and bisphenol-A by headspace solid-phase microextraction–gas chromatography–mass spectrometry in water samples and industrial effluents. *Int. J. Environ. Anal. Chem.* 90, 230–244.
- Mahugo Santana, C., Sosa Ferrera, Z., Esther Torres Padrón, M., Santana Rodriguez, J.J., 2009. Methodologies for the extraction of phenolic compounds from environmental samples: new approaches. *Molecules* 14, 298–320.
- Michalowicz, J., Duda, W., 2007. Phenols – sources and toxicity. *Polish. J. Environ. Stud.* 16, 347–362.
- Opeolu, B.O., Fatoki, O.S., Odendaal, J., 2007. Development of a solid-phase extraction method followed by HPLC–UV detection for the determination of phenols in water. *Int. J. Phys. Sci.* 2, 576–581.
- Padilla-Sánchez, J.A., Plaza-Bolaños, P., Romero-González, R., Barco-Bonilla, N., Martínez-Vidal, J.L., Garrido-Frenich, A., 2011. Simultaneous analysis of chlorophenols, alkylphenols, nitrophenols and cresols in wastewater effluents, using solid phase extraction and further determination by gas chromatography–tandem mass spectrometry. *Talanta* 85, 2397–2404.
- Patsias, J., Papadopoulou-Mourkidou, E., 2000. Development of an automated on-line solid-phase extraction–high-performance liquid

- chromatographic method for the analysis of aniline, phenol, caffeine and various selected substituted aniline and phenol compounds in aqueous matrices. *J. Chromatogr. A* 904, 171–188.
- Peng, J.F., Liu, J.F., Hu, X.L., Jiang, G.B., 2007. Direct determination of chlorophenols in environmental water samples by hollow fiber supported ionic liquid membrane extraction coupled with high-performance liquid chromatography. *J. Chromatogr. A* 1139, 165–170.
- Perret, D., Marchese, S., Sergi, M., Gentili, A., Notari, S., 2004. Hybrid quadrupole time-of-flight for the determination of chlorophenols in surface water by liquid chromatography–andem mass spectrometry. *Chromatographia* 59, 411–417.
- Puig, P., Borrull, F., Calull, M., Aguilar, C., 2008. Sorbent pre-concentration procedures coupled to capillary electrophoresis for environmental and biological applications. *Anal. Chim. Acta* 616, 1–18.
- Quintana, J.B., Rodil, R., Muniategui-Lorenzo, S., López-Mahía, P., Prada-Rodríguez, D., 2007. Multiresidue analysis of acidic and polar organic contaminants in water samples by stir-bar sorptive extraction–liquid desorption–gas chromatography–mass spectrometry. *J. Chromatogr. A* 1174, 27–39.
- Quintana, M.C., Ramos, L., 2008. Sample preparation for the determination of chlorophenols. *Trends Anal. Chem.* 27, 418–436.
- Regueiro, J., Becerril, E., Garcia-Jares, C., Llompart, M., 2009. Trace analysis of parabens, triclosan and related chlorophenols in water by headspace solid-phase microextraction with in situ derivatization and gas chromatography–tandem mass spectrometry. *J. Chromatogr. A* 1216, 4693–4702.
- Santana, C.M., Padrón, M.E., Ferrera, Z.S., Rodríguez, J.J., 2007. Development of a solid-phase microextraction method with micellar desorption for the determination of chlorophenols in water samples. Comparison with conventional solid-phase microextraction method. *J. Chromatogr. A* 1140, 13–20.
- Sarafraz-Yazdi, A., Dizavandi, Z.R., Amiri, A., 2012. Determination of phenolic compounds in water and urine samples using solid-phase microextraction based on sol–gel technique prior to GC-FID. *Anal. Method* 4, 4316–4325.
- Wada, M., Kinoshita, S., Itayama, Y., Kuroda, N., Nakashima, K., 1999. Sensitive high-performance liquid chromatographic determination with fluorescence detection of phenol and chlorophenols with 4-(4,5-diphenyl-1H-imidazol-2-yl)benzoyl chloride as a labeling reagent. *J. Chromatogr. B* 72, 179–186.
- Wennrich, L., Popp, P., Moder, M., 2000. Determination of chlorophenols in soils using accelerated solvent extraction combined with solid-phase microextraction. *Anal. Chem.* 72, 546–551.